## Sustained in vitro intestinal epithelial culture within a Wnt-dependent stem cell niche.

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**Public Summary:**
The in vitro analysis of intestinal epithelium has been hampered by a lack of suitable culture systems. Here we describe robust long-term methodology for small and large intestinal culture, incorporating an air-liquid interface and underlying stromal elements. These cultures showed prolonged intestinal epithelial expansion as sphere-like organoids with proliferation and multilineage differentiation. The Wnt growth factor family positively regulates proliferation of the intestinal epithelium in vivo. Accordingly, culture growth was inhibited by the Wnt antagonist Dickkopf-1 (Dkk1) and markedly stimulated by a fusion protein between the Wnt agonist R-spondin-1 and immunoglobulin Fc (RSpo1-Fc). Furthermore, treatment with the gamma-secretase inhibitor dibenzazepine and neurogenin-3 overexpression induced goblet cell and enteroendocrine cell differentiation, respectively, consistent with endogenous Notch signaling and lineage plasticity. Epithelial cells derived from both leucine-rich repeat-containing G protein-coupled receptor-5-positive (Lgr5(+)) and B lymphoma moloney murine leukemia virus insertion region homolog-1-positive (Bmi1(+)) lineages, representing putative intestinal stem cell (ISC) populations, were present in vitro and were expanded by treatment with RSpo1-Fc; this increased number of Lgr5(+) cells upon RSpo1-Fc treatment was subsequently confirmed in vivo. Our results indicate successful long-term intestinal culture within a microenvironment accurately recapitulating the Wnt- and Notch-dependent ISC niche.

**Scientific Abstract:**
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